membrane damage) or are we dealing with a new proton transporter was open. In this work, TCP-C15 was shown to slowly incorporate into membrane. Therefore, by long incubation (10–20 min) we showed an authentic uncoupling effect (stimulation of mitochondrial respiration) with nanomolar concentrations of this compound. With the help of a new experimental method, it was shown that the mitotropic compound of SkQ set, which in reductive conditions in the mitochondrion transforms into the hydroquinone form, shows uncoupling effect at nanomolar concentrations.

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3P.5 Establishing the role of the mitochondrial carrier MTCH2

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Mitochondrial outer-membrane permeabilization (MOMP), leading to cytochrome *c* release and caspase protease activation is a major event during apoptosis. BCL-2 family members are pivotal regulators of MOMP, but it is unknown how they regulate this process. BID, a pro-apoptotic BCL-2 family member, plays a critical role in executing apoptosis in the liver by initiating MOMP. Previously, mitochondrial carrier homolog 2 (MTCH2) was demonstrated to interact with BID in cells signaled to die. MTCH2 is a novel and previously uncharacterized 33-kDa protein, which belongs to the mitochondrial carrier family. By using a conditional knockout mouse MTCH2 was demonstrated to be essential for BID-induced apoptosis in the liver, but its mechanism of action remains unknown. A detailed bioinformatic analysis was carried out to assess how closely related MTCH2 is to other mitochondrial carriers. MTCH2 has the same structural features and topology as other mitochondrial carriers, but it has a unique substrate binding site, suggesting that the putative substrates differ chemically and structurally from known carrier substrates. In addition, MTCH2 has incomplete matrix and cytoplasmic salt bridge networks, indicating a low energy barrier to conformational changes. How will the substrates of MTCH2 be identified? The observation that MTCH2 has a unique substrate binding site with well-defined chemical features may help to narrow down substrate candidates. In addition, MTCH2 orthologs do not exist in yeast and plants, indicating that the substrate either does not exist or does not need to be transported into mitochondria in these organisms. To screen for substrate candidates the human MTCH2 was expressed in the cytoplasmic membrane of the Gram-positive bacterium *Lactococcus lactis*. The main advantage of using this system is that transport assays for the identification of the substrate can be performed with whole cells, as the carrier is directly accessible to the provided substrates. Establishing the role of MTCH2 as a mitochondrial carrier may have important implications for mitochondrial metabolism and the regulation of MOMP by BCL-2 family members during apoptosis.

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3P.6 Identification and characterization of functional residues in a multi-subunit type Na^+/H^+ antiporter Mrp complex from alkaliphilic *Bacillus pseudofirmus* OF4

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Mrp antiporters catalyze secondary $Na^+(Li^+)/H^+$ antiport and/or K^+/H^+ antiport that is physiologically important in diverse bacteria. Mrp is unique among antiporters in requiring all six or seven hydrophobic gene products (MrpA to MrpG) of the *mrp* operon for antiport activity. The MrpA, D and C proteins of the MrpA-B-C-D module have sequence similarity with Complex I subunits whereas the MrpE-F-G module is Mrp-specific [1,2]. A panel of site-direct mutants in 28 conserved or specific motif-related amino acid residues from an alkaliphile Mrp Na^+/H^+ antiporter was constructed. Each mutant transporter was expressed in antiporter-deficient *Escherichia coli* strain KNabc and the mutations were classified into 7 groups based on assays of growth/sodium-resistance, antiport properties, Mrp subunit levels, and formation of monomeric and dimeric Mrp complexes that are the active forms [1]. The analyses identified charged residues that are important for antiport activity and that are conserved across the large Mrp subunits of the MrpA-D module, MrpA, MrpD, as well as membrane-bound subunits (NuoL/M/N) of complex I. These included MrpA-K223, -K299 and MrpD-K219 as well as acidic residues that had been identified in *Bacillus subtilis* Mrp [3]. This study also extended evidence for a key role for MrpE of the MrpE-F-G module. MrpE is required for normal membrane levels of other Mrp proteins and normal complex formation. Conversely, some mutations in the MrpA-to-D module affected membrane levels of MrpE. Residues that are required for formation of the monomeric form or both forms of hetero-oligomeric Mrp complexes were identified for the first time. A mutation of Proline81 in MrpG produced a novel Mrp that supported sodium-resistance but lacked antiport activity. While a pair of tyrosines and a VFF motif with proposed roles in sodium-binding were mutated without effect, mutation of MrpA-H700 of a putative quinone binding site affected K_m values for the activity.

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3P.7 Effect of cardiolipin on the iron uptake of F_1F_OATP synthase in heart mitochondria

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Excessive iron is known to amplify ROS by Fenton and Haber–Weiss reaction, with subsequent damage of proteins, lipids and DNA of mitochondria. As one of the components affected by iron, cardiolipin, a tetra-acyl phospholipid, is crucial for oxidative phosphorylation of mitochondria. Recently, we showed that iron uptake was

associated with ATP hydrolytic activity in mitochondria. Since the activity of F_1F_OATP synthase is modulated by cardiolipin, we supposed that the cardiolipin might as well regulate the iron uptake in mitochondria. To study this postulation, proteoliposome reconstituted with iron overloaded enzyme was prepared. In the absence of cardiolipin but with asolectin only, iron uptake was stimulated more by ATP (1 mM) than ADP, whereas in the presence of cardiolipin, iron uptake was augmented higher by ADP (0.1 mM) than ATP. However, this ADP stimulation was diminished with augmenting the concentration of inorganic phosphate. Interestingly proteoliposome with iron overloaded enzyme exhibited parallel uptake of calcium and iron by ADP in the absence of cardiolipin. However, in the presence of cardiolipin, uptake pattern was changed as import of iron accompanies export of calcium and vice versa. This result suggests that cardiolipin may support iron uptake for iron overloaded enzyme by modulating molecular structure of enzyme.

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3P.8 Proton/ion co-transport by protein M2 of influenza virus A

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The M2 proton channel of influenza virus A was reconstituted into liposomes using our improved detergent-removal methodology (see Sindra Peterson Årsköld's poster). This yielded tight unilamellar vesicles of reproducibly high quality and unidirectional orientation of M2, with the N-terminal out. Using the novel, membrane-impermeable pH sensor Glu3 (Leiding *et al.*, 2009, *Anal. Biochem.* 388: 296-305), we monitored intravesicular pH and thus proton flux through M2. The observed flux was amantadine-sensitive, verifying that it was indeed channel-mediated. We present the proton flux recorded through M2 under a number of conditions: under a pH gradient in the absence and presence of valinomycin, under K^+(in)/Na^+(out) concentration gradients at varying pH, and under K^+(out)/Na^+(in) concentration gradients. We also show that while acidic reconstitution conditions inhibit M2 irreversibly, lowering the pH after reconstitution at pH 7 has a fundamentally different effect. Our results point towards M2 being a co-transporter rather than a pure proton channel: it can transport Na^+ along with H^+, it can transport Na^+ and K^+ in exchange for H^+, and it can transport H^+ in both directions. While not disputing the strong preference M2 shows for protons, our results explain how M2 acidifies the viral interior without building up an electrical counterpotential, and unifies a number of apparently contradictory results in the literature.

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3P.9 Studying the mechanisms of RNA translocation into mitochondria

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A growing field of evidence confirms that a large number of human diseases, such as mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) or myoclonic epilepsy with ragged red fibers (MERRF), arise from mutations in tRNA genes coded by the mitochondrial DNA [1]. An understanding of the fine mechanisms of tRNA import from cytosol could help us to improve delivery of "therapeutic" RNA molecules into mitochondria to cure the negative effects of these mutations at the molecular level. It has been demonstrated that the import of $tRNA^{Lys}$ into mitochondria of the yeast *Saccharomyces cervisiae* needs ATP as energy source and the electrochemical membrane potential $\Delta\psi$ [2] as a driving force for its transmembrane movement. It has also been shown that this $tRNA^{Lys}$ has to be aminoacylated in the cytosol by the lysyl-tRNA-synthetase (KRS) and that its import requires two cytosolic targeting factors—the enolase-2, an enzyme of the glycolytic pathway, and the cytosolic precursor of the mitochondrial lysyl-tRNA-synthetase (preMSK) [3,4]. Little is still known about the molecular mechanisms involved in the translocation of the $tRNA^{Lys}$ across mitochondrial membranes, and proteins implicated in its import are not identified yet. Since import of $tRNA^{Lys}$ requires the preMSK, their co-import through the pre-protein import machinery may be suggested. However, analysis of yeast strains, carrying deletions of non-essential genes coding for proteins of the pre-protein import machinery, revealed that none of these mutations had an effect on $tRNA^{Lys}$ import. With the help of Northwestern analysis, combined with tandem mass spectrometry (MALDI TOF), several proteins, among which mitochondrial porin, TOM40, adenine nucleotide translocator, HSP60 and subunit 2 of bc1 complex were identified as potential binders of $tRNA^{Lys}$ upon its import into mitochondria, and their possible impact on import was studied in comparison for wild type and Δ*POR1* yeast strain.

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3P.10 Visualization of cargo dynamics in COPII vesicle formation on artificial planar lipid membrane

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Intracellular vesicular transport in eukaryotic cell is composed of three steps; selected cargo proteins are loaded into 50–100 nm diameter membrane vesicles, cargo loaded vesicle bud from the surface of intracellular organelles and fuse with the membrane of the recipient organelles. Thereby, the membrane components of transport vesicles and cargo proteins are transported. These transport vesicles are coated with a protein complex called "coat protein", their binding is controlled by low molecular weight GTPase [1]. Coat protein complex II (COPII) vesicles are coated with the COPII coat proteins, namely, Sec23/24p and Sec13/31p and a low-molecular-weight GTPase Sar1p. The formation of the COPII vesicles starts when Sar1p, which is a low molecular weight GTPase, is converted from a GDP form (inactive form) to a GTP form (active form) by the guanine nucleotide exchange factor (GEF) Sec12p, which is present on the endoplasmic reticulum. COPII vesicle formation can be reconstituted *in vitro* by using these 5 protein complexes [2,3]. So, we try to